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Proposed Registration Decision PRD2013-14

Bacillus subtilis strain GB03

(publié aussi en français)

26 June 2013

This document is published by the Health Canada Pest Management Regulatory Agency. For further information, please contact:

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Canada

ISSN: 1925-0878 (print)
1925-0886 (online)

Catalogue number: H113-9/2013-14E (print version)
H113-9/2013-14E-PDF (PDF version)

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Overview

Proposed Registration Decision for *Bacillus subtilis* strain GB03

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Bacillus subtilis* GB03 Technical Fungicide and the associated end-use products, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, containing the technical grade active ingredient *Bacillus subtilis* strain GB03, for use as seed treatments to suppress seed and root diseases caused by *Fusarium* spp. and *Rhizoctonia solani* on canola, mustard (oilseed and condiment), rapeseed, and legume vegetables including soybeans.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Bacillus subtilis* GB03 Technical Fungicide and the associated end-use products, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

Before making a final registration decision on *Bacillus subtilis* strain GB03, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Bacillus subtilis* strain GB03, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Bacillus subtilis* strain GB03?

Bacillus subtilis strain GB03 is a bacterium that is used as a microbial pest control agent (MPCA) to suppress damping-off and root rot on canola, rapeseed and mustard (oilseed and condiment); and suppress seedling blight and root rot on legume vegetables including soybeans. *Bacillus subtilis* is a soil bacterium that is distributed globally, and commonly recovered from water, soil, air and decomposing plant residues. Strain GB03 of *B. subtilis* was originally isolated from healthy foliage of Douglas fir in Australia.

Bacillus subtilis strain GB03 is a root colonizer that directly competes with potential root pathogens for nutrients and space at the surface of roots and by producing antifungal agents such as iturins. *Bacillus subtilis* strain GB03 also reduces disease by inducing the plant's natural defense mechanisms, a process called induced systemic resistance.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Health Considerations

Can Approved Uses of *Bacillus subtilis* Strain GB03 Affect Human Health?

Bacillus subtilis strain GB03 is unlikely to affect your health when Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide are used according to the label directions.

People could be exposed to *B. subtilis* strain GB03 when handling and applying Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When spores of *B. subtilis* strain GB03 were tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

Residues in Water and Food

Dietary risks from food and water are not of concern

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure that the food Canadians eat is safe.

Bacillus subtilis strain GB03 is a ubiquitous bacterium that is commonly found in soil. When *B. subtilis* strain GB03 was administered orally to rats, no signs of toxicity or disease were observed, and no metabolites of toxicological significance have been shown to be produced by this strain of *B. subtilis*. Although some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning, these strains demonstrated the ability to produce a highly heat-stable toxin that may be similar to a toxin produced by *Bacillus cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* strain GB03 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 1992. Therefore the establishment of an MRL is not required for *B. subtilis*

strain GB03. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks from Handling Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide

Occupational risks are not of concern when Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide are used according to label directions, which include protective measures.

Workers handling Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide can come into direct contact with *B. subtilis* strain GB03 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers exposed to the end-use product must wear waterproof gloves, long-sleeved shirts, long pants, goggles, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks.

For the bystander, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Bacillus subtilis* strain GB03 is Introduced into the Environment?

Environmental risks are not of concern.

Information available in the published literature on the environmental fate of *B. subtilis* strain GB03 suggests that, as a soil microorganism, it is likely that *B. subtilis* strain GB03 could survive in soil under suitable environmental conditions (i.e., type of soil, moisture, acidity levels, and temperature) at elevated levels. However, the populations of *B. subtilis* strain GB03 should return to naturally occurring levels over time.

Studies were conducted to determine the effects of *B. subtilis* strain GB03 on birds, and terrestrial plants. These studies showed that *B. subtilis* strain GB03 was not toxic or pathogenic to birds and terrestrial plants. Waivers for avian pulmonary testing, wild mammals, arthropods and non-arthropod invertebrates toxicity testing as well as for freshwater fish, estuarine and marine fish, aquatic arthropods, and aquatic plants were deemed acceptable to address the remaining environmental toxicological requirements. The rationales were based on the ubiquitous nature of *B. subtilis* in both soil and water whose level in the terrestrial and aquatic environment will not significantly increase as a result of the use of Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide as seed treatments; the toxicity profile of *B. subtilis* strain GB03 from laboratory animal studies; and a review of published literature which indicated a few reports of adverse effects to terrestrial organisms, and a lack of adverse effects to aquatic organisms from natural populations of *B. subtilis*.

In published literature, other strains of *B. subtilis* have been reported to cause infections in mammals, terrestrial insects and plants. However, these reports were few in number considering the large amount of published literature on this microorganism. Furthermore, these reports involved unusual strains, or select strains, of *B. subtilis* for which their ability to cause disease was not thoroughly investigated. There are no reports with *B. subtilis* strain GB03 in non-target organisms except for the intended pest.

Value Considerations

What Is the Value of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicides?

***Bacillus subtilis* strain GB03, the active ingredient in Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, suppresses certain seedling and root diseases on canola, rapeseed, mustard (oilseed and condiment) and legume vegetables including soybeans.**

Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, containing 5.0×10^{10} colony forming units (CFU)/mL or 5.5×10^{10} CFU/g *Bacillus subtilis* strain GB03, are products formulated as seed treatments to suppress seedling and root diseases caused by *Fusarium* spp. and *Rhizoctonia solani* on canola, rapeseed, mustard (oilseed and condiment) and legume vegetables including soybeans. Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide provide additional alternatives to disease management options for these diseases both in conventional agriculture as well as organic farming. When the Flowable Fungicide and Kodiak Concentrate Fungicide are used with a chemical seed treatment, the combination also contributes to resistance management by different modes of action.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed to large quantities of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including *B. subtilis* strain GB03, contain substances that are potential sensitizers. Therefore, anyone handling or applying Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide must wear waterproof gloves, long-sleeved shirts, long pants, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and

shoes plus socks. Due to the irritation potential identified for Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, workers and handlers are also required to wear eye goggles. Also, the signal words, "POTENTIAL SENSITIZER" and "WARNING-EYE and SKIN IRRITANT" on the principal display panel and precautionary statements, "Causes eye and skin irritation. DO NOT get in eyes or on skin" and "May cause sensitization" are required on the secondary display panel of the label for Kodiak Concentrate Fungicide. The signal words, "POTENTIAL SENSITIZER" and "CAUTION-EYE and WARNING - SKIN IRRITANT" on the principal display panel and precautionary statements, "May irritate eyes. Avoid contact with eyes. Causes skin irritation. DO NOT get on skin" and "May cause sensitization" are required on the secondary display panel of the label for Kodiak Flowable Fungicide.

Environment

The end-use product labels will include environmental precaution statements that prevent the contamination of aquatic systems from the use of Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide.

Next Steps

Before making a final registration decision on *Bacillus subtilis* strain GB03, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Bacillus subtilis* strain GB03 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Bacillus subtilis strain GB03

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Bacillus subtilis* strain GB03
Function	To suppress seed and root diseases caused by *Fusarium* spp. and *Rhizoctonia solani* on canola, mustard (oilseed and condiment) and rapeseed, legume vegetables (Crop Group 6) including soybeans.
Binomial name	*Bacillus subtilis* strain GB03
Taxonomic designation[1]	
Kingdom	Eubacteria
Phylum	Firmicuties
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	*Bacillus*
Species group	*subtilis* group
Species	*subtilis*
Strain	GB03
Patent Status information	*Bacillus subtilis* strain GB03 had patent protection in Canada until 26 January 2013. Patent No. CA 2,129,239.
Minimum purity of active	*Bacillus subtilis* GB03 Technical Fungicide (technical grade active ingredient): 6.5×10^{11} colony forming units (CFU)/g Kodiak Flowable Fungicide (end-use product): 5.0×10^{10} CFU/mL Kodiak Concentrate Fungicide (end-use product): 5.5×10^{10} CFU/g
Identity of relevant impurities of toxicological, environmental and/or significance	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Bacillus subtilis* strain GB03 is not known to produce any toxic secondary metabolites (see Section 3.0).

[1] Taxonomy browser at: http://www.ncbi.nlm.nih.gov/pubmed/

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Grade Active Ingredient: *Bacillus subtilis* GB03 Technical Fungicide
End-Use Products: Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide

Properties	*Bacillus subtilis* GB03 Technical Fungicide	Kodiak Flowable Fungicide	Kodiak Concentrate Fungicide
Physical state	Homogeneous powder	Liquid (suspension)	Homogeneous powder
Colour	Tan	Brown	Beige
Odour	Musty/earthy	Rotted material (strong)	Rotted material (light)
pH (1% w/v)	4.8	5.13	8.51
Bulk Density	0.521 g/mL	1.1 g/mL	Loose: 0.468 g/mL Tapped: 0.525 g/mL
Guarantee	$\geq 6.5\times10^{11}$ CFU/g; 70% w/w	5.0×10^{10} CFU/mL; 7.3% w/w	$\geq 5.5\times10^{10}$ CFU/g; 50.6% w/w
Corrosion Character	None	None	Not Provided
Viscosity	Not applicable	289–320 cps	Not applicable

1.3 Directions for Use

Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide are biological seed treatments containing the rhizobacterium *Bacillus subtilis* strain GB03. To suppress damping-off and root rot caused by *Fusarium* spp. and *Rhizoctonia solani* on canola, rapeseed and mustard (oilseed and condiment), apply Kodiak Flowable Fungicide at the rates of 50 - 250 mL/100 kg seed or Kodiak Concentrate Fungicide at the rates of 45 - 225 g/100 kg seed. To suppress seedling blight and root rot caused by *Fusarium* spp. and *R. solani* on legume vegetables including soybeans, apply Kodiak Flowable Fungicide at the rate of 16 mL/100 kg seed or Kodiak Concentrate Fungicide at the rate of 7.75 g/100 kg seed. These end-use products may be used in combination with other registered chemical seed treatments for labeled crops and diseases.

1.4 Mode of Action

Bacillus subtilis strain GB03 acts as a biological control agent through two possible ways. The bacterial colonies establish quickly on host root surfaces by competing for space and nutrients with potential fungal root pathogens and inhibiting pathogens' ability to thrive and reproduce. The bacterium may also produce an antifungal peptide (iturins) that exhibits a wide suppressive spectrum to various pathogenic fungi, and acts against pathogens by competition and antibiosis.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Bacillus subtilis strain GB03 can be identified to the species level using a combination of colony morphologies on agar media and biochemical tests. Strain GB03 can also be identified to the strain level using a DNA-based method (for example, RiboPrinter). In addition, strain GB03 produces a light orange to buff pigment which can help distinguish this strain of *B. subtilis* strain GB03 from other naturally occurring strains.

2.2 Methods for Establishment of Purity of Seed Stock

A stock culture of *B. subtilis* strain GB03 is maintained in the American Type Culture Collection. Stock cultures are kept frozen at –80°C.

Practices for ensuring the purity of the seed stock were adequately described in the method of manufacture and quality assurance program.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The potency of the technical grade active ingredient and the end-use products will be determined by plate counting on agar media.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As noted in Section 2.1, the microbial pest control agent (MPCA) can be identified to the species level using a combination of colony morphologies on agar media and biochemical tests. Strain GB03 can be identified to the strain level using a proprietary DNA-based method, RiboPrinter. No methods are required to quantify viable or non-viable residues of *B. subtilis* strain GB03. *Bacillus subtilis* is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments. According to the United States Food and Drug Administration, some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat-stable toxin that may be similar to the vomiting type toxin produced by *Bacillus cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* strain GB03 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 1992. Furthermore, when *B. subtilis* strain GB03 was administered orally to rats, no signs of toxicity or disease were observed.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of *Bacillus subtilis* GB03 Technical Fungicide, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide are largely acceptable, however, the protocol to screen each batch of technical grade active ingredient for *Salmonella* spp. was found to be insufficient. The protocol must be amended to include an enrichment step to increase the sensitivity of the assay.

During manufacturing, several approaches will be used to limit microbial contamination in the technical grade active ingredient and end-use products. These approaches will include purity checks using microscopic techniques, and plating on selective agar media, sterilization of all equipment and media, and sanitization of recovery equipment.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of 3–4 production batches of product using microbe-specific screening methods for detecting and enumerating microbial contaminants of concern. However, representative batch screening data for the *Salmonella* enrichment method will be required for the technical grade active ingredient. Release standards for microbial contaminants comply with those permitted by the PMRA and are adequate to ensure that the end-use products do not contain unacceptable levels of human and animal disease-causing microorganisms.

No known toxic metabolites or hazardous substances are present in Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Results from storage stability testing of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide showed that these products are stable when stored at 4–25°C for a period of up to 1 year. No storage stability data are required for *Bacillus subtilis* GB03 Technical Fungicide since it is not stored as a separate product.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

A survey of published literature has revealed a number of instances where other *B. subtilis* strains had been implicated in infections in humans as well as the causal agent in food poisonings. Postoperative cellulitis, septicemia, respiratory disease, endocarditis and pneumonia have been reported in humans. In many instances, the association of *B. subtilis* is not sufficiently rigid for it to be regarded unequivocally as the causative agent. Also, the number of putative infections is extremely low considering the total number of reports of bacterial infections. Many of those cases involved drug abuses or severely debilitated patients. As *B. subtilis* is ubiquitous in the environment, it is expected that *B. subtilis* may sometimes be found in association with other microorganisms in infections. Only individuals treated with immunosuppressive drugs appear to be susceptible to infection from *B. subtilis*. In food borne illnesses, the United States

Food and Drug Administration has noted that some strains of *B. subtilis* have been isolated from food implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat stable toxin that may be similar to the vomiting type toxin produced by *B. cereus*, a known food borne pathogenic microorganism. *Bacillus subtilis* strain GB03 is not reported to produce this toxin. No such illnesses were reported for this microorganism in the United States where it has been registered for use on crops since 1992.

In other mammals, *B. subtilis* has been implicated in cases of bovine mastitis and reproductive disorders in goats. In the cases of bovine mastitis, *B. subtilis* could not be excluded as the causative agent. In goats exhibiting reproductive problems, high bacterial loads in infected vaginas were found to correlate with clinical symptoms. However, *B. subtilis* isolated from infected tissue was not pathogenic to Swiss white mice.

Bacillus subtilis has been reported to produce small antibiotic peptides and peptidolipids that are predominantly active against Gram positive bacteria but also against Gram negative bacteria, yeast and fungi. These include intracellular peptidolipids (mycosubtilin), extracellular cyclic peptidolipids (*Aspergillus* factor, bacillomycin A/B/D/F/L/R/S, eumycin, fengycin, iturin A and toximycin) and extracellular cyclic peptides (chaetomacin, fungistatin, mycobacillin and *Rhizoctonia* factor). Hemolytic activity has been reported for some peptidolipids.

A detailed review of the toxicological database for *Bacillus subtilis* strain GB03 was conducted. The database is complete, consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, intravenous infectivity, acute dermal toxicity/irritation and eye irritation) required for health hazard assessment purposes which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. These toxicology studies were conducted using a different technical grade active ingredient called GUS2000 that was determined to be equivalent to *Bacillus subtilis* GB03 Technical Fungicide. Unless otherwise stated, the test substance in the following toxicity studies was GUS2000.

Additional toxicity testing (acute oral toxicity, dermal irritation, and eye irritation) was performed on Companion, an end-use product that contains the same microbial pest control agent (MPCA) but a different formulation than Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of *B. subtilis* strain GB03 Technical Fungicide and its associated end-use products.

In an acute oral toxicity/pathogenicity study, no significant toxicity, no infectivity and no pathogenicity were observed in Sprague-Dawley rats following oral gavage with 1.9×10^8 CFU/animal of *B. subtilis* strain GB03. The test substance was initially detected only in lymph nodes and fecal matter, and not in brain, blood, kidney, spleen, liver nor lungs. By Day 4, the test substance was only isolated from the fecal matter. Based on the results of this study, *B. subtilis* strain GB03 is of low toxicity and is not pathogenic/infective in the rat when challenged via the oral route.

In a pulmonary toxicity/pathogenicity study, no significant toxicity was observed in Sprague-Dawley rats following intratracheal treatment with spores of *B. subtilis* strain GB03 at a dose greater than 2.84×10^8 viable spores per animal. By Day 15, all organs except the lungs were cleared of microbial counts. Although mean microbial counts were obtained by Day 36 in the lungs, the counts did diminish significantly by the end of the study period. At necropsy, lungs from some of the animals showed lesions, spots and congestion throughout the study period. These reactions were attributed to strong but normal immunological reactions. No treatment related effects were observed. Based on the results of this study, *B. subtilis* strain GB03 is of low toxicity and is not pathogenic/infective in the rat when challenged via the pulmonary route.

In an intravenous infectivity study, no mortalities and no treatment related clinical signs, necropsy findings or changes in body weights and organs were observed in Sprague-Dawley rats following injection with a dose of 1.82×10^7 CFU of *B. subtilis* strain GB03/animal in water. The microbe was cleared from brain, blood and caecum contents by Day 15, from kidney by Day 22. Although microbial colonies were obtained throughout the 36-Day study in the lymph node, spleen, liver and lungs, there was a significant decrease in the number of colonies by Day 36 when compared to the amount initially isolated at Day 2 (i.e. ranged from 100-fold to 1000-fold decrease). Based on the results, there was no evidence of pathogenicity observed in rats following intravenous injection with a dose of *B. subtilis* strain GB03 at 1.82×10^7 CFU/animal.

In an acute dermal toxicity study, no mortalities and no signs of toxicity were observed in New Zealand rabbits treated with *B. subtilis* strain GB03 at a dose of 1000 mg/kg body weight over 10% of the total body surface for 24 hours.

In an eye irritation study, irritation of the conjunctivae and cornea was observed one hour after 0.1 g of the ground *B. subtilis* strain GB03 was instilled into the conjunctival sac of the left eye of New Zealand white rabbits. Irritation was completely resolved in the cornea by Day 2 but not in the conjunctiva until Day 7 of the treatment period. Ground *B. subtilis* strain GB03 is moderately irritating to the eye based on the maximum irritation score (MIS) of 17/110 (at 1 hour) and the length of time it took for the irritation to completely dissipate. Consequently, "WARNING-EYE IRRITANT" and "Causes eye irritation. DO NOT get in eyes" are required on the principal and secondary panels, respectively for the *Bacillus subtilis* GB03 Technical Fungicide and Kodiak Concentrate Fungicide.

In other studies submitted, the tested substance was Companion. An acute oral toxicity study showed Companion to have low toxicity. A dermal irritation study showed Companion to be moderately irritating with an MIS of 3.5 (1 hour). An eye irritation study showed Companion to be non to minimally/slightly irritating with an MIS of 7.67/110 (1 hour). Although Companion contains the same MPCA, its formulation is significantly different from Kodiak Flowable Fungicide. As a result, these studies are of limited utility in the risk assessments. Based on a review of the formulants in Companion, the irritation observed was most likely due to the spores of *B. subtilis* strain GB03. No replacement studies are required but the signal words, "WARNING – SKIN IRRITANT" and "Causes skin irritation. DO NOT get on skin" are required on the primary and secondary panels, respectively for *Bacillus subtilis* GB03 Technical Fungicide and Kodiak Concentrate Fungicide. The signal words "WARNING- SKIN IRRITANT. CAUTION- EYE IRRITANT" and "Causes skin irritation. DO NOT get on skin.

May irritate eyes. Avoid contact with eyes" are required on the principal and secondary panels, respectively, for Kodiak Flowable Fungicide.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *B. subtilis* strain GB03 has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *B. subtilis* strain GB03.

Results of the toxicity and infectivity of *Bacillus subtilis* GB03 Technical Fungicide (*B. subtilis* strain GB03) and its associated end-use products, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, are summarised in Appendix I, Table 1.

3.2 Occupational / Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal and/or inhalation. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus subtilis* has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, dermal toxicity studies in animals demonstrated no signs of systemic toxicity to *B. subtilis* strain GB03.

The toxicity testing with the B. subtilis strain GB03 showed no toxicity or infectivity via the oral, dermal, pulmonary, intravenous routes of exposure. The submitted dermal and ocular irritation studies indicated that Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide cause skin and eye irritation. Therefore, precautionary label statements to not get the end-use product on the skin and in the eyes and use of proper clothing and PPE, such as waterproof gloves and protective eye-wear are required to mitigate occupational exposure concerns.

Although dermal toxicity or toxicity from inhalation exposure is considered minimal from the proposed use, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, goggles, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, and no significant occupational risks are anticipated for this product.

3.2.2 Bystander

Overall, the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the MPCA and the assumption that precautionary label statements will be followed by commercial applicators in the use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide.

The end-use product label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to adults, infants and children is expected to be negligible.

3.3 Incident Reports Related to Human and Animal Health

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.healthcanada.gc.ca/pesticideincident. Incidents from Canada and the United States were searched and reviewed for *Bacillus subtilis* strain GB03.

As of 2 January 2013, there have been no incidents related to health or the environment reported to the PMRA, nor summarized by the United States Environmental Protection Agency or the California Department of Pesticide regulation, for products containing *Bacillus subtilis* strain GB03.

3.4 Dietary Exposure and Risk Assessment

3.4.1 Food

The proposed use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide as seed treatments is not expected to result in any dietary exposure concern from *B. subtilis* strain GB03 because bacteria, such as *B. subtilis* are ubiquitous organisms found in most terrestrial environments.

Although the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children, or animals because *B. subtilis* strain GB03 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity study. Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or

pathogenicity in the test animals treated in the Tier I acute oral and pulmonary and subcutaneous injection toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.4.2 Drinking Water

The likelihood of *B. subtilis* strain GB03 entering neighbouring aquatic environments or surface water run-off from the use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide as seed treatments is considered very low.

No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The end-use product label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested not to allow effluent containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water. Therefore, potential exposure to *B. subtilis* strain GB03 in surface and drinking water is negligible.

3.4.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on the available information and hazard data, the PMRA concludes that *B. subtilis* strain GB03 is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCAs, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCAs and other registered micro-organisms that have a common mechanism of toxicity, does not apply to these MPCAs. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *B. subtilis* strain GB03 to human health.

3.5 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Bacillus subtilis are ubiquitous organisms found in most terrestrial environments. Residues of *B. subtilis* strain GB03 on treated food crops, at the time of harvest, are also anticipated as the active ingredient is comprised of endospores, which are much more persistent in the environment than vegetative cells. Consequently, the PMRA has applied a hazard-based approach for determining whether an MRL is required for this microorganism. Based on the lack of toxicity and pathogenicity effects observed in the acute toxicity and infectivity studies (particularly the oral study) and the fact that strain GB03 is not known to produce any mammalian toxins, the risks anticipated for dietary exposure are considered low. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that an MRL does not need to be established for *B. subtilis* strain GB03.

Bacillus subtilis strain GB03 is exempt from the requirement of a food tolerance in the United States.

3.6 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. subtilis* strain GB03 to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be very low since the product is to be used in agricultural sites and is not allowed for use on turf, residential or recreational areas. Furthermore, few adverse effects from exposure to *B. subtilis* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, there should not be any increase in potential human health risk.

3.7 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *B. subtilis* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *B. subtilis* strain GB03. No cumulative effects are anticipated if the residues of *B. subtilis* strain GB03 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *B. subtilis* strain GB03. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in nontarget organisms are noted in Tier I testing.

Although no environmental fate studies were submitted, basic information on the biological properties of *B. subtilis* was available in the public literature. *Bacillus subtilis* is a rod-shaped, Gram positive, aerobic, motile (peritichous flagella) bacterium that is ubiquitous in nature. *Bacillus subtilis* is commonly found in soil and in plant litter where they play an important role in the biological cycling of carbon and nitrogen. *Bacillus subtilis* is also found in various other habitats such as fresh water, polluted seawater, deep-sea sediments, foods, milk, pharmaceuticals, etc., usually as a result of environmental contamination (i.e. from soil, dust or colonized plant materials). The primary habitat of *B. subtilis* is considered to be soil. This species commonly produces proteases and other enzymes that breakdown various macromolecules for growth. Under adverse environmental conditions, *B. subtilis* produces an endospore that allows it to endure extreme conditions of heat and desiccation.

The proposed application of Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide as a seed treatment, and the subsequent planting of treated seeds into soil is not expected to result in a sustained increase of populations of the MPCA beyond those of naturally occurring soil dwelling *Bacillus* species found in the environment.

4.2 Effects on Non-Target Species

The PMRA has a four-level tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration (MCC) of the MPCA. The MCC is generally derived from the amount of the MPCA or its toxin expected to be available following application at the maximum recommended label rate multiplied by uncertainty factors. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies, i.e., life cycle studies, as well as definitive toxicity testing, for example, LC_{50} and LD_{50}. Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "conservative" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms. The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level risk quotient is below the LOC, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the LOC, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

The toxicity of *B. subtilis* strain GB03 to non-target organisms are summarised in Appendix I, Table 2.

4.2.1 Risks to Terrestrial Organisms

An avian oral toxicity study and a terrestrial plant phytotoxicity study were submitted to address the risks of *B. subtilis* strain GB03 to terrestrial organisms. Waivers for avian pulmonary testing, wild mammals, arthropods and non-arthropod invertebrates toxicity testing were deemed acceptable to address the remaining environmental toxicological requirements. The rationales were based on the ubiquitous nature of *B. subtilis* in both soil and water whose level in the

terrestrial environment will not significantly increase as a result of the use of Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide as seed treatments; the toxicity profile of *B. subtilis* strain GB03 from laboratory animal studies; and a review of published literature which indicated a few reports of adverse effects to terrestrial organisms.

There is the potential for oral exposure to birds from the proposed seed treatment use, as birds may consume food items that may have been exposed to seeds treated with *B. subtilis* strain GB03. The potential for the MPCA to cause acute oral toxicity and pathogenicity in an avian species was assessed in a study with Northern Bobwhite quail (*Colinus virginianus*). Three groups of healthy 21-day-old Northern Bobwhite quails were administered daily in the crop or proventriculus for five days with either *B. subtilis* strain GB03 (GUS2000, 3333 mg/kg bw/day; approximately 8.0×10^{10} spores/kg bw/day), water-soluble metabolites of *B. subtilis* strain GB03 (240 mg/kg bw/day) or washed spores of *B. subtilis* strain GB03 (2766 mg/kg bw/day; approximately 8.0×10^{10} spores/kg bw/day) in deionized water and observed over a total period of 30 days. A fourth group was administered daily for 5 days with deionized water at 10 mL/kg bw/day and served as a negative control group. In addition, each pen also included an untreated bird that served as an infectivity control. No treatment-related signs of toxicity or pathogenicity were noted throughout the study period. Gross necropsy results did not indicate any treatment related effects in either toxicity or pathogenicity. Consequently, *B. subtilis* strain GB03, water-soluble metabolites of *B. subtilis* strain GB03 and washed spores of *B. subtilis* strain GB03 are considered to be of low toxicity and not pathogenic to the Northern Bobwhite quail. There were also no reports of adverse effects to avian species from natural populations of *B. subtilis* in the published literature.

The potential hazard to non-target terrestrial plants was addressed in the plant toxicity and pathogenicity study, where seeds of *Glycine max* cv. Asgrow A-3427 were treated with *B. subtilis* strain GB03 in 1% sodium alginate at rates corresponding to 10^7, 10^5 and 10^3 viable spores per seed. Treated seeds were dried then incubated for 8 days at 30°C, 35°C or 40°C in germinator kimpac box systems to determine the effect of *B. subtilis* strain GB03 on the appearance of seedlings using a grading key developed by the United States Department of Agriculture. Untreated seeds and seeds treated with sodium alginate were similarly treated then allowed to germinate. Treatment with *B. subtilis* strain GB03 had no effect on the number of normal seedlings under the conditions of this study. The only effect noted was a temperature-dependent effect where a reduction in normal seedlings was observed at 35°C compared to 30°C and no normal seedlings were produced at 40°C in any of the treatments. This study demonstrated that *B. subtilis* strain GB03 was not pathogenic or toxic to seeds of *G. max* cv. Asgrow cultivar A-3427. References were also provided in the study report that implicated *B. subtilis* as the causal agent for *Bacillus* seed decay on soybean. These reports were all made from one laboratory that no longer had the *B. subtilis* cultures essential to in the original publications. A representative of the laboratory has indicated that the identity of the cultures involved was probably *B. megaterium* rather than *B. subtilis*.

Requests to waive avian pulmonary, wild mammal, arthropod and non-arthropod invertebrate toxicity testing were accepted based on the biological properties of the MPCA, anticipated levels of exposure and the frequency of reported effects in published literature. *Bacillus subtilis* is a ubiquitous microorganism in soil and the use of Kodiak Concentrate Fungicide and Kodiak

Flowable Fungicide as a seed treatment is not expected to considerably increase the natural background level of the microorganism in the terrestrial environment. Therefore the level of exposure of *B. subtilis* strain GB03 to non-target terrestrial organisms is expected to be minimal. In particular, exposure to birds by the pulmonary route is expected to be minimal. There were, however, a few reports in the published literature of adverse effects in mammals. These reports implicated *B. subtilis* in cases of bovine mastitis and reproductive disorders in goats. No other reports of adverse effects were reported to mammals, despite this microorganism's ubiquitous nature in the environment. Furthermore, the laboratory animal studies on the rat submitted in support of this registration indicate that there is no pathogenicity or infectivity to mammals via the oral, pulmonary or intravenous routes of exposure. In insects, some isolates of *B. subtilis* have been shown to be capable of infecting and causing mortality in mosquito larvae. Despite the ubiquitous nature of *B. subtilis*, few reports of adverse effects to insects were reported in published literature. *Bacillus subtilis* is not generally considered to be a pathogen of insects.

For earthworms and other soil macroorganisms, published literature revealed one report on the use of *B. subtilis* strain VM 132 as a potential biological control agent of the parasitic root-knot nematode (*Meloidogyne incognita*) of tomato. Plants grown from inoculated seed displayed significantly fewer nematode galls; however, the mode of action was not elucidated, and the pathogenic relationship was not established. In contrast, another published study demonstrated that *B. subtilis* displayed no toxicity or pathogenicity to the nematode, *Caenorhabditis elegans*, when fed clinical isolates of *B. subtilis*. *Bacillus subtilis* is generally not considered to be a pathogen of non-arthropod invertebrates. Although the product is intended to control pest microorganisms, *B. subtilis* is a normal component of the soil, and is not expected to affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes.

Based on all the available information on the effects of *B. subtilis* strain GB03 to terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods, terrestrial non-arthropod invertebrates, terrestrial plants and microorganisms from the proposed use of Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide.

4.2.2 Risks to Aquatic Organisms

Requests to waive the requirement for toxicity testing of freshwater fish, estuarine and marine fish, aquatic arthropods, and aquatic plants were accepted based on the biological properties of *B. subtilis* strain GB03, the proposed use of Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide, and the frequency of adverse effects in the published literature. The use of Kodiak Concentrate Fungicide and Kodiak Biological Fungicide will be limited to seed treatment application. This intended use pattern minimizes direct exposure to non-target aquatic organisms. Given the expected levels of *B. subtilis* strain GB03 on the treated seeds, the amount of *B. subtilis* strain GB03 reaching aquatic environments from leaching or runoff is expected to be minimal. Nevertheless, the biological properties of this microorganism suggest that spores of this MPCA could survive in aquatic ecosystems. However, no harm to aquatic organisms are expected based on the absence of disease or other adverse effects in fish or other aquatic organisms related to *B. subtilis* in published literature despite the ubiquitous nature of the

microorganism. Instead, *B. subtilis* was studied as a possible probiotic in numerous animals, including the gilthead seabream (*Sparus aurata* L.) and tiger shrimp (*Panaeus monodon*). Although these studies were clearly not designed to assess potential toxicity and pathogenicity of *B. subtilis*, the results showed no obvious adverse effects.

Based on all the available data and information on the effects of *Bacillus subtilis* strain GB03 to aquatic organisms, there is reasonable certainty that no harm will be caused to non-target aquatic organisms from the proposed seed treatment of Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide. As a general precaution, label statements will be added to the label requiring handlers to not contaminate irrigation or drinking water or aquatic habitats by cleaning of equipment or disposal of wastes.

4.3 Incident Reports related to the Environment

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.healthcanada.gc.ca/pesticideincident. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; American causality of highly probable, probable and possible) are considered in the reviews.

As of 8 January 2013, there were no environmental incidents reported in the PMRA Incident reporting database or in the United States Environmental Protection Agency's Ecological Incident Information System for products containing *B. subtilis* for use as pesticides.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

5.1.1.1 Suppression of damping-off and root rot caused by *Fusarium* spp. and *Rhizoctonia solani* on canola, rapeseed and mustard (oilseed and condiment)

Eighteen trials were submitted and reviewed to support the proposed claim, including 16 trials on canola (three laboratory studies, six small-plot field trials and seven demonstration strip trials) and two field trials on mustard. The efficacy data from laboratory studies, small-plot field trials and large-scale field trials demonstrated that Kodiak FL or Kodiak Concentrate Fungicide suppressed the seed and seedling diseases caused by *Rhizoctonia solani* and *Fusarium* spp. on canola and mustard. For instance, the addition of Kodiak FL induced numerically higher stand counts than that of chemical seed treatment alone when Kodiak FL was applied in tank mix with a chemical seed treatment in all small-plot field trials. In the large-scale demonstration trials, the addition of Kodiak FL increased stand counts by an average of 5% and seed yield by 2% or 8% on average compared to that of chemical seed treatment alone.

Based on efficacy data and scientific rationale, the claim of Kodiak Flowable Fungicide or Kodiak Concentrate Fungicide to suppress damping-off and root rot caused by *Fusarium* spp. and *Rhizoctonia solani* on canola, rapeseed and mustard (oilseed and condiment) is supported according to the proposed use pattern.

5.1.1.2 Suppression of seedling blight and root rot caused by *Fusarium* spp. and *Rhizoctonia solani* on legume vegetables including soybeans

Data from four field trials on soybean, lentil and field pea were provided to support the use claim for suppression of seedling blight and root rot caused by *Fusarium* spp. and *R. solani*, using Kodiak Concentrate Fungicide alone or tank mixed with a chemical seed treatment. In two trials inoculated with *Fusarium* spp., Kodiak Concentrate Fungicide alone consistently increased the stand counts from 20% up to 142% and reduced seedling blight by 59% to 73% on various assessments in the lentil trial, and both Kodiak Concentrate Fungicide alone and Kodiak Concentrate Fungicide tank mixed with a conventional fungicide significantly reduced the tap root infection by 48% and 56% in the soybean trial, respectively. In two trials inoculated with *R. solani*, Kodiak Concentrate Fungicide alone and Kodiak Concentrate Fungicide plus a conventional fungicide reduced the root rot severity on the tap root by 40–73% and 52–81%, respectively. The results can be extrapolated to other legume vegetables in Crop Group 6 since the same pathogens affect other legumes in the same manner in terms of pathogen biology, pathogen/crop interaction and crop biology.

Based on efficacy data and scientific rationale, the claim of Kodiak Flowable Fungicide or Kodiak Concentrate Fungicide to suppress seedling blight and root rot caused by *Fusarium* spp. and *Rhizoctonia solani* on legume vegetables including soybeans is supported with a modification of the application rate.

5.2 Phytotoxicity to Host Plants

No phytotoxicity or crop injury was reported.

5.3 Economics

No market analysis was done for this submission. However, the registrant demonstrated economic benefits of using Kodiak Flowable Fungicide or Kodiak Concentrate Fungicide to suppress seed and seedling diseases on canola. In the small-plot field trials, applying Kodiak FL at 50 mL and 250 mL/100 kg seed produced on average an additional return of $18.91/ha and $63.45/ha, respectively. In the large-scale field trials, applying Kodiak FL at 50 mL/100 kg seed produced on average an additional return of $9.69/ha.

5.4 Sustainability

5.4.1 Survey of Alternatives

Refer to Appendix I, Table 3 for a summary of the active ingredients currently registered for the same uses as Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide.

5.4.2 Compatibility with Current Management Practices Including Integrated Pest Management

Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide can be combined with certain conventional seed treatments to control or suppress the major seed and seedling diseases of the crops listed on the labels.

5.4.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Although the active ingredient *B. subtilis* is assigned as a Group 44 fungicide based on the FRAC guidelines, the development of resistance to *B. subtilis* strain GB03 is not a concern at this time since part of the mode of action is related to competitive exclusion, and it is assumed that there is a very low risk of developing resistant strains among *Rhizoctonia* and *Fusarium* populations. *Bacillus subtilis* strain GB03 establishes itself quickly on root surfaces thereby competing with potential fungal root pathogens.

5.4.4 Contribution to Risk Reduction and Sustainability

Rhizoctonia and *Fusarium* are common soil-borne pathogens that cause various seed and seedling diseases on a wide range of plant hosts. These diseases lead to reductions in crop stand and ultimately in yield. Consequently, treatment of seed has become normal practice and is considered as a grower's best line of defence against seed and seedling diseases. Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide are biological fungicides whose mode of action is partly based on competitive growth and exclusion of pathogens on target crops. The use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide will contribute to an integrated pest management program for the proposed crops.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy, which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

While reviewing *Bacillus subtilis* strain GB03 Technical Fungicide, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in the technical product, *Bacillus subtilis* strain GB03 Technical Fungicide, and formulants in the end-use products, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide. The PMRA has reached the following conclusions:

Bacillus subtilis strain GB03 Technical Fungicide does not meet the Track 1 criteria because the active ingredients is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track 1 criteria.

Therefore, the use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide is not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants and Contaminants of Health or Environmental Concern

Bacillus subtilis strain *GB03* Technical Fungicide does not contain any formulants of health or environmental concern identified in the Canada Gazette, Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern (Amended 25 June 2008 SI\2008-67). There are also no formulants or contaminants of health or environmental concern present in the associated end-use products, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *Bacillus subtilis* GB03 Technical Fungicide, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide were deemed adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications of the end-use products were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of one year when stored at 4–25°C. However, the protocol to screen each batch of technical grade active ingredient for *Salmonella* spp. must be amended to include an enrichment step to increase the sensitivity of the assay. Also, representative batch screening data for this *Salmonella* enrichment method will be required for the technical grade active ingredient.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *B. subtilis* strain GB03 were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests, spores of *B. subtilis* strain GB03 were of low toxicity by the oral, pulmonary, intravenous and dermal routes, and they were not pathogenic or infective via the oral, pulmonary and intravenous injection exposure routes in animals. The technical grade active ingredient and the end-use products have the potential to irritate eyes and skin, and they are considered to be potential sensitizers.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation.

Label statements (i.e. Potential Sensitizer, may cause sensitization) and risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, goggles, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal.

7.3 Environmental Risk

The non-target organism testing, scientific rationales and supporting published scientific literature submitted in support of *Bacillus subtilis* strain GB03 Technical Fungicide, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide were determined to be sufficiently complete. The use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide is not expected to pose a risk to birds, mammals, arthropods, fish, and plants when the directions for use on the label are followed. No other environmental fate studies or non-target organism studies are required to assess the risk of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide used as a commercial-class biological fungicide to suppress seed and root diseases caused by *Fusarium* spp. and *Rhizoctonia* spp.

As a specific precaution, the Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide label instructs users to not contaminate irrigation or drinking water supplies or aquatic habitats by application of product, cleaning of equipment or disposal of wastes.

7.4 Value

Value information was provided to support the use of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide to suppress seedling and root diseases caused by *Fusarium* spp. and *Rhizoctonia solani* on canola, rapeseed, oilseed mustard and legume vegetables including soybeans. Other seed treatments are currently registered in Canada for control or suppression of *Rhizoctonia* and *Fusarium* on seeds and seedlings; however, the registration of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide presents an additional alternative to disease management options for these diseases both in conventional agriculture as well as organic farming. The combination of conventional seed treatments and of Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide also provides protection to the root for a longer time than with chemicals alone. The chemical partner acts quickly and helps get the seedling established while Kodiak Concentrate Fungicide and Kodiak Flowable Fungicide provide extended protection of seedlings. The combination of both a chemical and biological component contributes to resistance management by different modes of action.

A summary of the proposed and accepted uses for Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide is presented in Appendix I, Table 4.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Bacillus subtilis* GB03 Technical Fungicide and the associated end-use products, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide, containing the technical grade active ingredient *Bacillus subtilis* strain GB03, for use as seed treatments to suppress seed and root diseases caused by Fusarium spp. and Rhizoctonia solani on canola, mustard (oilseed and condiment), rapeseed, and legume vegetables including soybeans.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

°C	degree(s) Celsius
ADI	acceptable daily intake
ARD	acute reference dose
bw	bodyweight
CFU	colony forming unit
cps	centipoise
DNA	deoxyribonucleic acid
EC_{50}	effective concentration on 50% of the population
FL	Flowable
FRAC	Fungicide Resistance Action Committee
g	gram
kg	kilogram
L	litre
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
mg	milligram
MIS	maximum irritation score
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute for Occupational Safety and Health
NOEL	no observed effect level
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
TSMP	Toxic Substances Management Policy
v/w	volume per weight

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Bacillus subtilis* GB03 Technical Fungicide (*B. subtilis* strain GB03) and its associated end-use products, Kodiak Flowable Fungicide and Kodiak Concentrate Fungicide

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Toxicity/Infectivity of GUS2000 (form of technical grade active ingredient)				
Acute Oral Infectivity and Toxicity (21-Day study)	Rat- Sprague-Dawley 15/sex, 1.9×10^8 CFU/animal, Interim sacrifices on Days 2, 8, 15, 22 (22-day study) 8/sex untreated control 2/sex shelf-control	$LD_{50} > 1.9 \times 10^8$ CFU/animal	**LOW TOXICITY** **NO PATHOGENICITY** **NO INFECTIVITY** **ACCEPTABLE**	PMRA 2103042
Pulmonary Infectivity and Toxicity (Intratracheal)	Rat- Sprague-Dawley 24/sex, 2.84×10^8 CFU/animal, interim sacrifices on Days 2, 4, 8, 15, 22 and 36 (36-day study). 5/sex untreated control 2/sex shelf-control	$LD_{50} > 2.84 \times 10^8$ CFU/animal	**LOW TOXICITY** **NO PATHOGENICITY** **NO INFECTIVITY** **ACCEPTABLE**	PMRA 2103048
Intravenous Infectivity	Rat- Sprague-Dawley 21/sex; 1.82×10^7 CFU/animal, interim sacrifices on Days 1, 2, 4, 8, 15, 22, and Day 36; (36-day study) Control: 6/sex; saline only	$LD_{50} > 1.82 \times 10^7$ CFU/animal	**NO PATHOGENICITY** **NO INFECTIVITY** **ACCEPTABLE**	PMRA 2103050

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Dermal Toxicity (GUS2000 is similar to the end-use product, Kodiak Concentrate Fungicide)	Rabbit- New Zealand white, 5/sex, undiluted, 1000 mg/kg bw to an area of approximately 10% of total body surface, exposed for 24 hours (14-day study) (2000 mg/animal)	LD_{50} > 1000mg/kg bw (3.6×10^9 CFU/animal)	**LOW TOXICITY ACCEPTABLE**	PMRA 2103052
Acute Irritation/Sensitization of GUS2000				
Study Type	**Species, Strain, and Doses**	**Results**	**Comments**	**Reference(s)**
Eye Irritation	Rabbit-New Zealand albino, 6 males, 0.1 g of the ground GUS2000 (equivalent to 2.2×10^{10} CFU/animal), conjunctival sac of left eye, instilled for entire study. Observed for 7 days after instillation	MIS = 17.17/110 (1 hour)	**MILDLY IRRITATING** **Label statements required:** **Principal: "CAUTION – EYE IRRITANT"** **Secondary: "May irritate eyes. Avoid contact with eyes."** **Or the next level up for label statements due to cornea irritation within the hour and eye irritation lasted 7 days** **ACCEPTABLE**	PMRA 2103054
Acute Toxicity of Companion				
Acute Oral Toxicity	Rat- Sprague-Dawley 5 males/5 females, 5g of Companion/kg bw (8.2×10^6 CFU/mL of *Bacillus subtilis* strain GB03)	LD_{50} > 5000mg/kg bw (3.4×10^7 CFU/kg bw) of *Bacillus subtilis* strain GB03	**Companion has low toxicity** **ACCEPTABLE study, but of limited utility in risk assessment due to test substance**	PMRA 2106389
Acute Irritation/Sensitization of Companion				
Dermal Irritation	Rabbit- New Zealand White, 3/sex, 0.5mL (8.2×10^6 CFU/mL of *Bacillus subtilis* strain GB03)	MIS = 3.5 (1 hour) (4.1×10^6 CFU/animal)	Moderately irritating **ACCEPTABLE study, but of limited utility in risk assessment due to test substance**	PMRA 2106391

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Eye Irritation	Rabbit- New Zealand White, 0.1 mL (8.2×10^6 CFU/mL of *Bacillus subtilis* strain GB03)	MIS = 7.67/110 Unwashed group (8.2×10^5 CFU/animal)	Non to minimally/slightly irritating **ACCEPTABLE study, but of limited utility in risk assessment due to test substance**	PMRA 2106390

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effects, Comments	Reference(s)
Terrestrial Organisms				
		Vertebrates		
Birds	*Colinus virginianus* (21 days old) 30-day oral	Birds (30) were gavaged with *B. subtilis* strain GB03, (technical grade active ingredient) at a dose of 3333 mg/kg bw/day or 8.0×10^{10} sp/kg bw for 5 consecutive days. A control group (10 birds) was gavaged with washed spores of *B. subtilis* strain GB03 (technical grade active ingredient) at a dose of 2766 mg/kg/bw or approximately 8.0×10^{10} sp/kg bw for 5 consecutive days. A control group (10 birds) was gavaged with water soluble metabolites (spore filtrate) at a dose of 667 mg/kg bw for 5 consecutive days. Negative control group (10 birds) was dosed with deionized water at 0.1% (v/w) for 5 consecutive days. Infectivity control, 12 birds (no dosing administered)	No treatment related mortalities or overt signs of toxicity were reported in the treatment groups. There were no signs of pathogenicity or infectivity. 30-day acute oral $LD_{50} > 8.0 \times 10^{10}$ sp/kg bw for 5 consecutive days. 30-day NOEL 8.0×10^{10} sp/kg bw for 5 consecutive days. **ACCEPTABLE**	PMRA 2103058
	Pulmonary	A request to waive the requirement for test data was submitted based on the scientific rationale that pulmonary or inhalation exposure is expected to be minimal with the		PMRA 2103056 PMRA

Organism	Exposure	Protocol	Significant Effects, Comments	Reference(s)
		proposed used pattern and no toxicity or pathogenicity was observed in the avian oral study. Avian pulmonary testing was waived.		2103059
Wild Mammals	A request to waive the requirement for test data was submitted based on the scientific rationale that no toxicity or pathogenicity was observed in the acute mammalian studies submitted for human health and safety testing (see Section 3.1). *Bacillus subtilis* strain GB03 (technical grade active ingredient) was not toxic to mammals via the oral, pulmonary or dermal routes; they were also not pathogenic via the oral, pulmonary or intravenous route. No further data are required to assess the risk of harm to non-target wild mammals.			PMRA 2103056 PMRA 2103059
Invertebrates				
Arthropods	A request to waive the requirement for test data was submitted based on the scientific rationale that exposure is expected to be minimal and few reports of adverse effects to arthropods were found following a search of published scientific literature despite the ubiquitous nature of *B. subtilis*. No further data are required to assess the risk of harm to non-target arthropods.			PMRA 2103056 PMRA 2103059
Non-Arthropod Invertebrates	A request to waive the requirement for test data was submitted based on the scientific rationale that exposure is expected to be minimal and no reports of adverse effects to non-arthropod invertebrates were found following a search of published scientific literature. No further data are required to assess the risk of harm to non-target non-arthropod invertebrates.			PMRA 2103056 PMRA 2103059
Plants				
Terrestrial Plants	*Glycine max* cv. Asgrow A-3427	Seeds (250 g) were treated with *B. subtilis* strain GB03 at rates of 10^7, 10^5 and 10^3 CFU/seed. Observed for inhibitory effects on seed germination. Solvent control: seeds (250 g) were treated with 1% sodium alginate Untreated control: seeds (250 g) were untreated	No treatment-related effects. $EC_{50} > 10^7$ CFU/seed **ACCEPTABLE**	PMRA 2103061
Aquatic Organisms				
Vertebrates				
Freshwater Fish	A request to waive the requirement for test data was submitted based on the scientific rationale that exposure is expected to be minimal and no reports of adverse effects to freshwater fish were found following a search of published scientific literature. No further data are required to assess the risk of harm to non-target freshwater fish.			PMRA 2103056 PMRA 2103059

Organism	Exposure	Protocol	Significant Effects, Comments	Reference(s)
Invertebrates				
Aquatic Arthropods	A request to waive the requirement for test data was submitted based on the scientific rationale that exposure is expected to be minimal and no reports of adverse effects to aquatic arthropods were found following a search of published scientific literature. No further data are required to assess the risk of harm to non-target aquatic arthropods.			PMRA 2103056 PMRA 2103059
Plants				
Aquatic Plants	A request to waive the requirement for test data was submitted based on the scientific rationale that exposure is expected to be minimal and no reports of adverse effects to aquatic plants were found following a search of published scientific literature. No further data are required to assess the risk of harm to non-target aquatic plants.			PMRA 2103056 PMRA 2103059

Table 3 Summary of Alternatives for the Same Uses as Kodiak Flowable and Kodiak Concentrate Fungicides

Crop	Disease	Active ingredient and FRAC Fungicide Group	
		Conventional	**Non-conventional**
Canola, rapeseed and mustard (oilseed and condiment)	Damping-off and root rot caused by *Rhizoctonia solani* and *Fusarium* spp.	Carbathiin (7) + thiram (M3); Difenoconazole (3) + metalaxyl (4) + fludioxonil (12); Fludioxonil (12); Metalaxyl (4) + carbathiin (7) + trifloxystrobin (11); Metalaxyl (4) + penflufen (7) + trifloxystrobin (11); metalaxyl (4) + carbathiin (7) + thiram (M3); Ipconazole (3) + carbathiin (7); Iprodione (2) + thiram (M3); Thiram (M3); Trifloxystrobin (11)	*Bacillus subtillis* MBI 600 (44)
Legume vegetables including soybeans	Seedling blight and root rot caused by *Rhizoctonia solani* and *Fusarium* spp.	Carbathiin (7) + thiram (M3); Metalaxyl (4) + azoxystrobin (11) + fludioxonil (12); Fludioxonil (12); Metalaxyl (4) + fludioxonil (12); Metalaxyl (4) + trifloxystrobin (11); Penflufen (7) + trifloxystrobin (11); Thiabendazole (1) + carbathiin (7); Thiabendazole (1) + metalaxyl (4) + fludioxonil (12); Thiram (M3); Trifloxystrobin (11)	*Bacillus subtillis* QST 713 (44)

Table 4 Use (Label) Claims Proposed by Applicant and Accepted

Proposed claim	Accepted claim
1) Suppression of root diseases caused by *Rhizoctonia* spp. and *Fusarium* spp. at the rates of 50 - 250 mL/100 kg seed for Kodiak Flowable Fungicide or 45 - 225 g/100 kg seed for Kodiak Concentrate Fungicide on canola, rapeseed and mustard (oilseed and condiment).	Accepted with modified disease name of "damping-off and root rot caused by *Rhizoctonia solani* and *Fusarium* spp."
2) Suppression of root diseases caused by *Rhizoctonia* spp. and *Fusarium* spp. at the rates of 16 mL/100 kg seed for Kodiak Flowable Fungicide or 7.75 - 31.25 g/100 kg seed for Kodiak Concentrate Fungicide on legume vegetables including soybeans.	Accepted with: a) modified application rate for Kodiak Concentrate at 7.75 g/100 kg seed only; b) modified disease name of "seedling blight and root rot caused by *Rhizoctonia solani* and *Fusarium* spp."
3) Tank mixtures with other registered Bayer CropScience seed treatment fungicides and insecticides such as Trilex AL Concentrate, Prosper FX, PENCLOTRIME, PENTRI, PENPROME, Raxil MD, Raxil Pro MD for crops which they are labelled.	Accepted as proposed.

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

The Active Ingredient, Its Properties and Uses

2103026 DACO: M1.1
2103064 2011, Product Profile and Proposed Use M1.2-1.3, DACO: M1.2,M1.3
2289887 DACO: 1.1.2,M1.1
2289889 DACO: 1.1.2,M1.1
2289893 DACO: 1.1.2,M1.1

Methods of Analysis

2103034 1998, Test methods of alternate supplier for GUS 2000 (EPA Reg. No. 7501-144) and GUS 376 (EPA Reg. No. 7501-147), DACO: M2.10.2,M2.10.3 CBI
2103035 1991, Stability data - GUS 2000 concentrate biological fungicide, DACO: M2.11 CBI
2103036 2004, The corrosion characteristics and storage stability of Kodiak concentrate end-use product, DACO: M2.11 CBI
2103037 1992, Product chemistry data - GUS 2000 concentrate, DACO: M2.11 CBI
2103038 2003, The physical characteristics of Kodiak concentrate end-use product, DACO: M2.12 CBI
2103039 1991, Product chemistry data - Gus 2000 concentrate biological fungicide, DACO: M2.10.1,M2.10.2,M2.10.3,M2.12,M2.7.1,M2.7.2,M2.8,M2.9.1,M2.9.2,M2.9.3 CBI
2103041 2003, Preliminary analysis of Kodiak concentrate, GB03, DACO: M2.10.1,M2.10.3,M2.9.2 CBI
2103063 Product Characterization M2.1 - 2.6., DACO: M2.1,M2.2,M2.3,M2.4,M2.5,M2.6
2103108 2004, The physical characteristics of Kodiak FL end-use-product, DACO: M2.12 CBI
2103109 2004, The corrosion characteristics and storage stability of Kodiak FL end use product, DACO: M2.11 CBI
2106312 DACO: M1.1
2106381 MSDS Osprey - GB03, DACO: M2.9.1 CBI
2106382 2011, GB03 History & Efficacy, DACO: M2.7,M2.7.1,M2.7.2 CBI
2106383 2010, Mfg Methods & Quality Assurance, DACO: 3.2.2,M2.8 CBI
2106384 2009, Methodology for Determining Unintentional Contaminants, DACO: M2.10.2,M2.8,M2.9.3 CBI
2106385 DACO #M2.10.1 Methodology for Active Ingredient, DACO: M2.10.1 CBI
2106386 2011, Product Specification Reports Midwest Laboratories, DACO: M2.12 CBI
2106387 2011, Identification and Enumeration of Culturable Bacteria by Water, DACO: M2.9.1,M2.9.2 CBI

2106392 2011, RiboPrinter Sample Report *Bacillus subtilis* GB03 Lot# 9536139-Osprey Bio, DACO: M2.7.1 CBI
2106393 2011, RiboPrinter Sample Report *Bacillus subtilis* GB03 Lot# R87 001 JD 275 - Bayer Kodiak, DACO: M2.7.1 CBI
2106394 2011, RiboPrinter Comparison, DACO: M2.7.1 CBI
2106395 Manufactures Information, DACO: M2.2
2106396 1995, Storage Stability Companion Biological Fungicide, DACO: M2.11 CBI
2106397 2009, Storage Stability Companion Biological Fungicide, DACO: M2.11 CBI
2106398 2011, Active Ingredient *Bacillus subtilis* Gram Positive Bacteria, DACO: M2.7 CBI
2108639 2011, Part 2.8: Manufacturing Methods and Quality Assurance, DACO: M2.8 CBI
2108640 2011, Part 2.9.2: Potency Estimation and Product Guarantee, DACO: M2.9.2 CBI
2108641 2011, Part 2.9.3: Unintentional Ingredients, DACO: M2.9.3 CBI
2108642 2011, Part 2.10.1: Active Ingredient or MPCA, DACO: M2.10.1 CBI
2108645 2011, Part 2.10.2: Analysis for Microbial Contamination, DACO: M2.10.2 CBI
2108649 2011, Part 2.10.3: Analysis for Other Unintentional Ingredients, DACO: M2.10.3 CBI
2200386 OBT M2.8-Kodiak Concentrate Fungicide, DACO: M2.12 CBI
2200387 OBT M2.12-Kodiak Fungicide Concentrate, DACO: M2.12 CBI
2200388 OBT M2.9.2-Kodiak-Concentrate-Fungicide, DACO: M2.9.2 CBI
2200389 OBTM2.9.3 - Kodiak Concentrate Fungicide, DACO: M2.9.3 CBI
2201644 2012, Summary of Physical and Chemical Properties, DACO: M2.12 CBI
2201646 2012, Product Characterization and Analysis - Quality Assurance (QA), DACO: M2.8 CBI
2201647 2012, Potency Guarantee and Product Guarantee, DACO: M2.9.2 CBI
2201649 2012, Unintentional Ingredients, DACO: M2.9.3 CBI
2201650 2012, Product Characterization and Analysis/Storage Stability, DACO: M2.11 CBI
2202160 2012, Manufacturing Processes, DACO: M2.8 CBI
2202163 2012, Product Specifications, DACO: M2.9.1 CBI
2202164 2012, Potency Estimation and Product Guarantee, DACO: M2.9.2 CBI
2202166 2012, Storage Stability, DACO: M2.11 CBI
2202168 2012, Summary of Physical and Chemical Properties, DACO: M2.12 CBI
2202172 2012, Unintentional Ingredients, DACO: M2.9.3 CBI

2.0 Human and Animal Health

2103029 M.4.3.3 Data Waiver Request, DACO: M4.1,M4.3.1,M4.3.3
2103030 M.4.7 Data Waiver Request, DACO: M4.1,M4.7
2103031 M.4.8 Data Waiver Request, DACO: M4.1,M4.8
2103032 M.5 Data Waiver Request, DACO: M5.0
2103033 M.7 Data Waiver Request, DACO: M7.0
2103036 2004, The Corrosion Characteristics and Storage Stability of Kodiak Concentrate End-Use Product, DACO: M2.11 CBI
2103037 1992, Product Chemistry Data - GUS 2000 Concentrate, DACO: M2.11 CBI

2103038 2003, The Physical Characteristics of Kodiak Concentrate End-Use Product, DACO: M2.12 CBI

2103039 1991, Product chemistry data - Gus 2000 concentrate biological fungicide, DACO: M2.10.1,M2.10.2,M2.10.3,M2.12,M2.7.1,M2.7.2,M2.8,M2.9.1,M2.9.2,M2.9.3 CBI

2103041 2003, Preliminary analysis of Kodiak concentrate, GB03, DACO: M2.10.1,M2.10.3,M2.9.2 CBI

2103042 1990, Acute oral toxicity/pathogenicity study of GUS2000 in rats, DACO: M4.1,M4.2.1,M4.2.2

2103048 1990, Acute pulmonary toxicity/pathogenicity study of GUS2000 in rats, DACO: M4.1,M4.2.1,M4.2.3

2103050 1990, Acute intravenous toxicity/pathogenicity study of GUS2000 in rats, DACO: M4.1,M4.3.1,M4.3.2

2103052 1990, Acute dermal toxicity test of GUS2000 in rabbits, DACO: M4.1,M4.4

2103054 1990, Primary eye irritation study of GUS2000 in rabbits, DACO: M4.3.1,M4.9

2103056 1991, Bacillus subtilis GB03 (ATCC no. SD-1397) - Petition for exemption from requirement for tolerance, DACO: M7.0

2103057 EPA, 1992, *Bacillus subtilis* GB03; exemption from the requirement of a tolerance, DACO: M7.0

3.0 Environment

2103056 1991, *Bacillus subtilis* GB03 (ATCC no. SD-1397) - Petition for exemption from requirement for tolerance, DACO: M7.0

2103058 1992, *Bacillus subtilis* strain GB03: An avian oral pathogenicity and toxicity study in the bobwhite, DACO: M9.2.1

2103059 1990, EEB review - Kodiak HB, Gus 376 concentrate / Gus 2000 concentrate, DACO: 9.3,9.4.1,9.4.2,9.5.1,9.5.2,9.6.2,M12.5,M9.2.1,M9.8.1,M9.8.2

2103061 1991, Evaluation of *Bacillus subtilis* GB03 (ATCC No. SD-1397) for its effect on soybeans (Glycine Max), DACO: M9.8.1

2103065 Summary of Environmental Toxicology Studies on *Bacillus subtilis* GB03, DACO: M9.1

2103087 2011, Kodiak flowable and Kodiak concentrate biological fungicides (*Bacillus subtilis* strain GB03), DACO: M10.1, M10.2.1, M10.2.2, M10.3.1, M10.3.2.1, M10.4.1, M10.4.2, M10.4.3, M10.4.4, M10.5

4.0 Value

2103110 2011, Kodiak flowable and Kodiak concentrate biological fungicides (*Bacillus subtilis* strain GB03), DACO: M10.1,M10.2.1,M10.2.2,M10.3.1,M10.3.2.1,M10.3.2.2,M10.4.1,M10.4.2,M10.4.3,M10.4.4,M10.5

2171004 10.2 Kodiak Biological Fungicides, DACO: M10.2

2171007 Tab 1 - SD09NARTAHGAM1 FUSASP, DACO: M10.2

2171008 Tab 2 - SD09NARTAHJLB2 FUSASP, DACO: M10.2

2171010 Tab 3 - SD09NARTAHGSH1 RHIZOC, DACO: M10.2

2171011 Tab 4 - SD09NARTAHJLB1 RHIZOC, DACO: M10.2

B. Additional Information Considered

i) Published Information

1.0 Environment

2292532 Li Nuo Liang, James L. Sinclair, Larry M. Mallory, and Martin Alexander, 1982, Fate in Model Ecosystems of Microbial Species of Potential Use in Genetic Engineering, DACO: M9.4.1, M9.5.1, M9.5.2, M9.6, M9.8.2

2292552 Danielle A. Garsin, Costi D. Sifr, Eleftherios Mylonakis, Xiang Qin, Kavindra V. Singh, Barbara E. Murray, Stephen B. Calderwood, and Frederick M. Ausubel, 2001, A simple model host for identifying Gram-positive virulence factors, DACO: M9.6, M9.8.2

2292553 Elena P. Ivanova, Mikhail V. Vysotskii, Vasilii I. Svetashev, Olga I. Nedashkovskaya, Natalia M. Gorshkova, Valery V. Mikhailov, Noboru Yumoto, Yasushi Shigeri, Takahisa Taguchi, Susumu Yoshikawa, DACO: M9.4.1, M9.5.1, M9.5.2, M9.6, M9.8.2.

2292556 S. Patnayak and A. Sree, 2005, Screening of bacterial associates of marine sponges for single cell oil and PUFA. 2005, S. Patnayak and A. Sree, DACO: M9.4.1, M9.5.1, M9.5.2, M9.6, M9.8.2.

2292574 Arellano-Carbajal, F., and Olmos-Soto, J., 2002, Thermostable a-1,4- and a-1,6-glucosidase enzymes from Bacillus sp. isolated from a marine environment , Arellano-Carbajal, F., and Olmos-Soto, J., DACO: M9.4.1, M9.5.1, M9.5.2, M9.6, M9.8.2